Relationship between Plasminogen Activator Inhibitor-1 (PAI-1) and Beta Fibrinogen Polymorphisms with Unexplained Recurrent Pregnancy Loss


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Abstract

Background: Recurrent Pregnancy Loss (RPL), is distressing for the patient and the cause is unknown in half of the cases and risk of it increases with the number of previous pregnancy losses.

Objective: The objective of this study was to identify the associations of the Plasminogen Activator Inhibitor-1 (PAI-1) and beta fibrinogen polymorphisms with Recurrent Pregnancy Loss (RPL).

Subjects and Methods: A cross-sectional, case-control study including 185 patients who had recurrent pregnancy loss as a patients group and 125 women who did not have any history of pregnancy loss as a control group. This study carried out at El-Hussein University Hospital in the period from November 2011 until October 2013.

Results: There was significant increase in PAI-1 4G/5G polymorphism in cases of recurrent pregnancy loss. Where there's significant difference in p-value, OR ratio of the PAI-1 for 4G/5G polymorphism between patients and controls where p-value and odds ratio of genotype 4G/5G was (0.019, 0.586) respectively and for 4G/4G were (0.023, 1.679) respectively, while, the p-value, OR ratio of 5G/5G genotype were (0.041, 1.449) respectively. Also, there's significant difference in frequency, p-value and OR ratio of the 4G alleless PAI-1 4G/5G polymorphism between patients and controls where the number and percentages of 4G alleles among patients and controls were 162 (43.78%) in the patients and 111 (44.4%) in controls respectively and for 5G allele were 208 (56.22%) in patient, 139 (55.6%) in controls. The p-value and OR ratio of both 4G and 5G alleles were the same (0.05, 0.975).

Also, there's significant difference in p-value, OR ratio of the genotype β-fibrinogen 455 G/A polymorphism between patients and controls. Where p-value and of genotype G/G were (0.005, 0.519) respectively and for A/A were (0.049, 1.623) respectively, while, the p-value, OR ratio of heterozygous G/A genotype were (0.024, 1.798) respectively. Also, there's significant difference in frequency, p-value and OR ratio of the alleless β-fibrinogen 455 G/A polymorphism between patients and controls where the number and present-

Conclusion: Our Study showed that there is significant association was observed between PAI-1 4G/5G polymorphism and B-Fibrinogen 455 G/A in one hand and RP1 on other hand.

Key Words: Recurrent pregnancy loss – Plasminogen Activator Inhibitor-1 (PAI-1) – Beta fibrinogen polymorphism.

Introduction

RECURRENT Pregnancy Loss (RPL), is distressing for the patient and the cause is unknown in half of the cases and risk of it increases with the number of previous pregnancy losses. Of clinically recognized pregnancies, 10-15% results in pregnancy loss, usually before 14 weeks of gestation. The chance of having a normal pregnancy is 30% in a woman who had three recurrent pregnancy losses, 25% after 4 losses [1].

Thrombophilia is defined as a disorder of hemostasis in which there is a tendency for the occurrence of thrombosis in veins or arteries due to abnormalities in blood composition, blood flow, or the vascular wall. The term thrombophilia is most often used in combination with venous thrombosis [2].

VTE is a complex common disease in which multiple risk factors both acquired and genetic, are involved in the development of the disease. Many acquired risk factors have been identified such as surgery, immobilization, oral contraceptive, pregnancy, malignancy, and advanced age [2].
Pregnancy is associated with a 20-200% increase in levels of fibrinogen and factors II, VII, VIII, X, and XII. In contrast, endogenous anticoagulant levels may increase minimally (tissue factor pathway inhibitor) or remain constant or significantly decrease (Protein S) in pregnancy. Moreover, levels of immunoreactive and functionally active PAI-1 increase up to three-fold in pregnancy [3].

Fibrinolysis serves to prevent excess clotting by breaking down the fibrin clot. Fibrinolysis is mediated by tissue-type plasminogen activator which binds to fibrin also fibrinolysis is inhibited by Plasminogen Activator Inhibitor (PAI-1) which is the fast in-activator of tissue type plasminogen activator [4].

Plasma PAI-1 concentrations have been related to a common guanosine insertion/deletion gene polymorphism, 4G/5G, 675 bp upstream from the start site of translation [5].

For successful implantation, invasion of the cytotrophoblast to the proper depth of the uterus is crucial. This provides anchorage for the conceptus and promotes adaptation of uteroplacental circulation. Plasminogen Activator Inhibitor 1 (PAI-1) is believed to control proteolysis and remodeling of maternal tissue during trophoblast invasion [6].

Elevated fibrinogen levels have been associated with increased risk for thromboembolism resulting in RPL. Fibrinogen is composed of three pairs of polypeptide chains (named \( \alpha \), \( \beta \) and \( \gamma \)) which are linked by disulfide bonds. These three chains are encoded by three different genes (\( \alpha \), \( \beta \) and \( \gamma \), respectively), that are located on chromosome 4 [7].

**Aim of the work:**

To identify the associations of the Plasminogen Activator Inhibitor-1 (PAI-1) and beta fibrinogen polymorphisms with Recurrent Pregnancy Loss (RPL).

**Subjects and Methods**

A cross-sectional, case-control study including 185 patients who had recurrent pregnancy loss as a patients group and 125 women who did not have any history of pregnancy loss as a control group. This study carried out at El-Hussein University hospital in the period from November 2011 until October 2013.

**Inclusion criteria:**

Patients in this study are selected according to:
- Age between 18-40 years.
- Three or more consecutive unexplained pregnancy loss.
- Gestational age at abortion below 12 weeks.
- No previous live births.

**Exclusion criteria:**

Patients having known or definitive cause(s) explaining their miscarriages were excluded from the study like:
- Anatomical uterine abnormalities.
- History of endocrinal disturbance as DM and hypothyroidism.
- History of autoimmune diseases or positive antiphospholipid antibodies.
- Known abnormal karyotyping of the couples.
- History of congenital anomalies in previous pregnancies.
- History of consanguinity between the patient and her husband.
- History of chronic diseases as cardiac, liver or kidney diseases.

**All patients in this study are subjected to:**
- Comprehensive history taking.
- Detailed examination.
- Pelvic ultrasound.
- All patients and controls were subjected for assay of DNA extraction and detection of PAI-1 and \( \beta \) fibrinogen gene mutations by PCR technique. After obtaining approval of local scientific and ethical committees as well as an informed consent from all cases and controls, four centimeters of maternal venous blood samples were collected from each subject by venipuncture, the blood preserved from clotting by adding EDTA to the samples and stored on refrigerator for 2 to 3 days before processing which include isolation of genomic DNA, PCR amplification and hybridization of amplified products.

Data were collected, revised and verified, data were then analyzed statistically using SPSS version 16 (SPSS, Chicago, IL, USA). The following tests were used:
- \( \text{MEAN} = X \).
- \( \text{Standard deviation} = \text{SD} \).
- For independent samples \( t \)-test was used.
- Pearson correlation coefficient (\( r \)).
- \( \text{Chi square test} = \chi^2 \).

**Results**

310 women included in this study where classified into: Patients group which included 185
cases who had a history of three or more unexplained recurrent abortions in their first trimester and control group which include 125 women who had two or more successful pregnancies and none of them had any history of fetal loss.

There was significant increase in PAI-1 4G/5G polymorphism in cases of recurrent pregnancy loss. Where there's significant difference in p-value, OR ratio of the PAI-1 for 4G/5G polymorphism between patients and controls where p-value and OR ratio of 4G/5G was (0.019, 0.586) respectively and for 4G/4G were (0.023, 1.679) respectively, while, the p-value, OR ratio of 5G/5G genotype were (0.041, 1.449) respectively. Also, there's significant difference in frequency, p-value and OR ratio of the 4G allele PAI-1 4G/5G polymorphism between patients and controls where the number and percentage of 4G alleles among patients and controls were 162 (43.78%) in the patients and 111 (44.4%) in controls respectively and for 5G allele were 208 (56.22%) in patient, 139 (55.6%) in controls. The p-value and OR ratio of both 4G and 5G alleles were the same (0.05, 0.975).

Also, there's significant difference in p value, OR ratio of the genotype β-fibrinogen 455 G/A polymorphism between patients and control. Where p-value and of genotype G/G were (0.005, 0.519) respectively and for A/A were (0.049, 1.623) respectively, while, the p-value, OR ratio of heterozygous G/A genotype were (0.024, 1.798) respectively. Also, there's significant difference in frequency, p-value and OR ratio of the alleles β-fibrinogen 455 G/A polymorphism between patients and controls where the number and percentages of G alleles among patients and controls were 275 (74.32%) in the patients and 208 (83.2%) in controls respectively and for A allele were 95 (25.68%) in patient, 42 (16.8%) in controls. p-value and OR ratio of both G and A allele was the same (0.009, 0.585).

Table (1): Comparison between the patients and controls regarding age groups.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Cases n=185</th>
<th>Controls n=125</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>2</td>
<td>1.6</td>
<td>0.19</td>
</tr>
<tr>
<td>20-25</td>
<td>35</td>
<td>38</td>
<td>0.71</td>
<td>0.48</td>
</tr>
<tr>
<td>26-30</td>
<td>76</td>
<td>41.1</td>
<td>45</td>
<td>0.36</td>
</tr>
<tr>
<td>31-35</td>
<td>40</td>
<td>21.6</td>
<td>30</td>
<td>0.24</td>
</tr>
<tr>
<td>&gt;35</td>
<td>10</td>
<td>5.4</td>
<td>13</td>
<td>10.4</td>
</tr>
</tbody>
</table>

Range 20-38 19-40 0.602 (NS)
Mean±S.D 26.72±5.0705 27.64±5.7146 0.550

Table (2): Number of abortions per patient.

<table>
<thead>
<tr>
<th>Number of abortions</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>13.5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table (3): Genotype frequency of PAI-1 4G/5G polymorphism among patients and control.

<table>
<thead>
<tr>
<th>Patients (n=185)</th>
<th>Control (n=125)</th>
<th>Odds ratio [C.I. 95%]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G/5G</td>
<td>106</td>
<td>57.30</td>
<td>0.586</td>
</tr>
<tr>
<td>4G/4G</td>
<td>28</td>
<td>15.14</td>
<td>1.679</td>
</tr>
<tr>
<td>5G/5G</td>
<td>51</td>
<td>27.57</td>
<td>1.449</td>
</tr>
</tbody>
</table>

Table (4): p-value, OR ratio of Genotype frequency of PAI-1 4G/5G polymorphism among patients and control.

<table>
<thead>
<tr>
<th>Patients (n=185)</th>
<th>Control (n=125)</th>
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<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G/5G</td>
<td>106</td>
<td>57.30</td>
<td>0.019</td>
</tr>
<tr>
<td>4G/4G</td>
<td>28</td>
<td>15.14</td>
<td>0.023</td>
</tr>
<tr>
<td>5G/5G</td>
<td>51</td>
<td>27.57</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Table (5): Frequency, p-value and OR ratio of PAI-1 4G/5G alleles polymorphism among patient and control.

<table>
<thead>
<tr>
<th>Patients (n=370)</th>
<th>Control (n=250)</th>
<th>Odds ratio [C.I. 95%]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G allele</td>
<td>162</td>
<td>43.78</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients (n=370)</th>
<th>Control (n=250)</th>
<th>Odds ratio [C.I. 95%]</th>
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</tr>
</thead>
<tbody>
<tr>
<td>4G allele</td>
<td>162</td>
<td>43.78</td>
<td>0.05</td>
</tr>
</tbody>
</table>
### Table (6): Genotype frequency of β fibrinogen 455 G/A polymorphism among patient and control.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n=185)</th>
<th>Control (n=125)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>G/G</td>
<td>104</td>
<td>56.2</td>
</tr>
<tr>
<td>G/A</td>
<td>67</td>
<td>36.2</td>
</tr>
<tr>
<td>A/A</td>
<td>14</td>
<td>7.6</td>
</tr>
</tbody>
</table>

### Table (7): p-value, OR ratio of genotype frequency of β fibrinogen 455 G/A polymorphism among patient and control.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n=185)</th>
<th>Control (n=125)</th>
<th>p-value</th>
<th>Odds ratio [C.I. 95%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>G/G</td>
<td>104</td>
<td>56.2</td>
<td>89</td>
<td>71.2</td>
</tr>
<tr>
<td>G/A</td>
<td>67</td>
<td>36.2</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>A/A</td>
<td>14</td>
<td>7.6</td>
<td>6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

### Table (8): Frequency, p-value and OR ratio of β fibrinogen alleles 455 G/A among patient and control.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n=370)</th>
<th>Control (n=250)</th>
<th>p-value</th>
<th>Odds ratio [C.I. 95%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>G allele</td>
<td>275</td>
<td>74.32</td>
<td>208</td>
<td>83.2</td>
</tr>
<tr>
<td>A allele</td>
<td>95</td>
<td>25.68</td>
<td>42</td>
<td>16.8</td>
</tr>
</tbody>
</table>

### Discussion

Couples with pregnancy loss need empathy and understanding. In our opinion, early pregnancy loss, especially when recurrent, is an emotionally traumatic experience, similar to that associated with stillbirth or neonatal death. The loss of clinically recognized pregnancies prior to the 20th week of gestation occurs at a frequency of 20% [8].

Studies suggest that, 4-6% of all women attempting pregnancy will experience at least two miscarriages, and about 1-2% will have 3 or more miscarriages [9].

Although several causes of RPL have been established, more than 50% of cases remain unexplained. Recently, thrombophilias have been suggested as a possible cause of RPL [10].

A normal pregnancy is dependent on adequate placental circulation and fetal vasculature. The development of a normal functioning vascular network requires complicated cooperation between different cell types and various growth factors in the processes of implantation, placentation and embryo development [11].

In pregnancy, there is an increase in fibrinogen, von Willebrand factor, and clotting factors II, VII, VIII, IX, and X. There is a decrease in physiologic anticoagulants manifested by a decrease in Protein S (PS) and an increase in Protein C (PC) resistance. There are decreases in fibrinolytic activity, increased venous stasis, and vascular injury associated with delivery. Pregnancy is associated with increased clotting potential, decreased anticoagulant activity, and decreased fibrinolysis. Genetic studies may provide clues for the evaluation of the importance of different, fibrinolytic, thrombophilic, immunogenic and angiogenic molecules in RPL [12].

Inherited thrombophilias can result from gene mutations involved in coagulation. During pregnancy, the thrombogenic potential of inherited disorders is enhanced because of the hypercoagulable state produced by normal pregnancy-associated changes in several coagulation factors [13].

This study was planned in order to check for the association of genetic mutations related to Plasminogen Activator Inhibitor-1 (PAI-1) β-fibrinogen with unexplained RPL among affected women.

Soltanghoraei and his co-workers, (2007), have also reported that patients with homozygote 4G mutation were significantly more prone to RPL (OR: 8.2, 95% CI: 1.8-36.5) [14].
In our study there was significant increase in PAI-1 4G/5G polymorphism in cases of recurrent pregnancy loss. Where there’s significant difference in p-value, OR ratio of the PAI-1 for 4G/5G polymorphism between patients and controls. Where p-value and odds ratio of genotype 4G/5G was (0.019, 0.586) respectively and for 4G/4G were (0.023, 1.679) respectively, while, the p-value, OR ratio of 5G/5G genotype were (0.041, 1.449) respectively. Also, there’s significant difference in frequency, p-value and OR ratio of the 4G allele PAI-1 4G/5G polymorphism between patients and controls where the number and percentages of 4G alleles among patients and controls were 162 (43.78%) in the patients and 111 (44.4%) in controls respectively and for 5G allele were 208 (56.22%) in patient, 139 (55.6%) in controls. The p-value and OR ratio of both 4G and 5G alleles were the same (0.05, 0.975).

The PAI-1 gene’s promoter region contains at least two alleles producing either a 4Gor 5Gbase-pair region. The 5G allele permits the binding of transcription factor inhibitors that suppress gene transcription. In contrast, the 4G allele is too small to permit the binding of gene repressors. Therefore, individuals homozygous for the 4G/4G allele have a three to five-fold higher level of circulating PAI-1 compared with those bearing the 5G/5G or 5G/4G alleles [15].

This is in agreement with Tehrani and his coworkers, (2011), who showed that mutations of the PAI-1 4G allele increased the risk of RPL occurrence (p<0.05) [16].

Confirmatory to our results, the recent study by Subrt and his colleagues, that demonstrated a strong positive association between PAI-1 and pregnancy loss [17].

Zonouzi and his co-workers, confirmed no relationship was founded between the presence of PAI-1 mutant alleles and recurrent pregnancy loss [18].

Also, Sun and his colleagues, confirmed that homozygosity for PAI-1 4G polymorphisms is significantly higher in patients with RPL, which may result in increased PAI-1 concentrations and hypofibrinolysis and contribute to early pregnancy loss [19].

Another demonstrated that the 4G variant of PAI-1 gene was present in about two-thirds of women with celiac disease who experienced early pregnancy loss, a frequency significantly higher than that observed in the control group. Thus, they suggested that the 4G variant of the PAI-1 gene may predispose a subset of celiac women to pregnancy loss [20].

Also, disagree with our results, Buchholz and his co-worker, stated that there was a tendency toward a more 4G/4G use among patients with RPL (39.1%) than that in controls (32.3%), but the difference was not significant (p=0.22) [21].

In addition to that, Gumpel and his colleagues, (2009), did not find any association between this polymorphism and early or late pregnancy loss [22]. Moreover, Goodman and co-workers, (2009), found no differences in the frequencies of heterozygous, homozygous, total abnormal, and normal PAI-1 mutations were observed when patients experiencing RPL were compared with control women in their study [23].

It may be suggested that higher plasma fibrinogen levels derived from G/A polymorphism may lead to increased intravascular fibrin deposition and promote placental thrombosis leading to recurrent pregnancy loss [16].

Our results signify the increase of β-fibrinogen 455 G/A mutation in cases of recurrent pregnancy loss. In accordance with tehhrani and his coworker, (2009) data that show the increased risk of RPL in association with β-fibrinogen-455 G/A polymorphism (p<0.001) [16].

Where there’s significant difference in p value, OR ratio of the genotype β-fibrinogen 455 G/A polymorphism between patients and controls. Where p-value and of genotype G/G were (0.005, 0.519) respectively and for A/A were (0.049, 1.623) respectively, while, the p-value, OR ratio of heterozygous G/A genotype were (0.024, 1.798) respectively. Also, there’s significant difference in frequency, p-value and OR ratio of the alleleless β-fibrinogen 455 G/A polymorphism between patients and controls where the number and percentages of G alleles among patients and controls were 275 (74.32%) in the patients and 208 (83.2%) in controls respectively and for A allele were 95 (25.68%) in patient, 42 (16.8%) in controls. p-value and OR ratio of both G and A allele was the same (0>009, 0.585).

In the other hand, Coulam and his co-worker, has been reported that whilst none of the specific thrombophilic gene mutations appear to be a risk factor for recurrent miscarriage on their own [24].

Also conversion of fibrinogen to insoluble fibrin plays a pivotal role in haemostatic balance and
exposure of its non-substrate thrombin-binding sites after fibrin clot formation promotes anti-thrombotic properties.

In contrary to our results, Ciacci and his colleagues, did not confirm any association between β-fibrinogen-455G/A with recurrent pregnancy loss [20].

Also, Mahmoud and his co-workers, found that there is no differences in the frequency of specific gene mutations were detected when women with recurrent miscarriage were compared with control women [26].

Beta Fibrinogen (BF) 455G/A promoter region polymorphism is associated with 7-10% higher plasma fibrinogen levels, which could enhance concentration driven enzyme-substrate interactions between thrombin, fibrinogen, and platelets and thus leads to increased intravascular fibrin deposition and promoting placental thrombosis [25].

**Conclusion:**

Our study performed at on 310 patients and we concluded that:

- The study showed that there is significant association was observed between PAI-1 4G/5G polymorphism and RPL.
- The study showed that there is significant association between the β-fibrinogen 455 G/A polymorphism and RPL.

**Recommendations:**

Performing further studies are needed to determine the relationship of PAI-1 and β-fibrinogen polymorphisms to calculate the prevalence of PAI-1 and β-fibrinogen polymorphisms on a larger sample in order to determine the actual prevalence and to signify the importance of adding these factors as ordinary screening tests for RPL in the Middle East and especially in Egypt.

**References**


